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BIOCHEMICAL CHANGES DURING RIPENING OF THE MANGO FRUIT

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Abstract—Changes in oxo acids, free amino acids and some related enzymes were studied during the respiratory climacteric of the mango fruit, *Mangifera indica*. Maximum concentration of α-oxoglutarate and pyruvate was reached earlier than the climacteric peak. Oxaloacetate was not found to accumulate as the fruit ripened. Aspartate and glutamate increased for about three days after harvest and their concentration decreased as the climacteric maximum was reached. γ-Aminobutyrate showed an increase during the same period. Activities of malic enzyme (L-malate; NADP oxidoreductase decarboxylating: 1.1.1.41) and L-aspartate-(oxoglutarate aminotransferase (2.6.1.1) followed the respiratory pattern of the fruit while L-glutamate 1-carboxylase (4.1.1.15) showed a gradual increase in activity. An attempt is made to explain these changes with reference to the climacteric behaviour of the fruit.

INTRODUCTION

MANY fruits are known to go through a process of climacteric rise during ripening.[1] This rise in respiration is regarded as involving synthetic activities probably concerned in the development of enzyme systems during ripening. Many of these activities have been followed by various workers in different fruits. In apples Hulme *et al.*[2] have suggested that the main cause of the onset of climacteric rise appears to be the increased activity of malic enzyme and pyruvic carboxylase. Rhodes and Wooltorton[3] have shown increased activity of certain hydrolytic enzymes during ripening of apples. In banana, Tager *et al.*[4] have shown carboxylase and aldolase activities during ripening. Some workers have studied the levels of oxo acids[5] and free amino acids[6] during storage. In the present investigation certain changes of this nature are followed with reference to the respiratory climacteric of the mango fruit (*Mangifera indica*). The study includes changes in the activities of three different enzymes and quantitative estimations of certain oxo acids and free amino acids.

RESULTS

The pattern of carbon dioxide release during the climacteric phase of the mango fruit is given in Fig. 1. Over a number of seasons it has been observed that the climacteric peak in this variety of mango is reached around eighth day after harvest when the fruit is stored at 28° and it is ripe and ready for table use by about the 12th day.

The results given in Table 1 for the 2:4 dinitrophenyl-hydrazones of the oxo acids prepared from the extracts of mango pulp provide strong evidence for the presence of pyruvic acid, oxaloacetic acid and α-oxoglutaric acid in the pulp. In confirmation, reduction of the phenylhydrazones of pyruvate and α-oxoglutarate yielded α-alanine and glutamic

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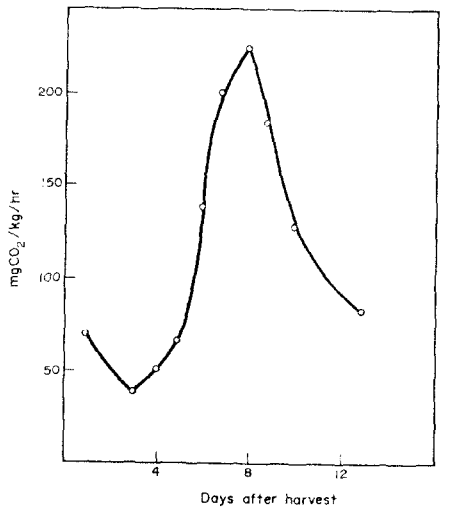


FIG. 1. RESPIRATORY PATTERN OF MANGO FRUIT (*Mangifera indica*) AT 28°. EACH VALUE REPRESENTS AVERAGE OF FOUR DIFFERENT ESTIMATIONS. FRUITS WERE PICKED ON 23–5–68.

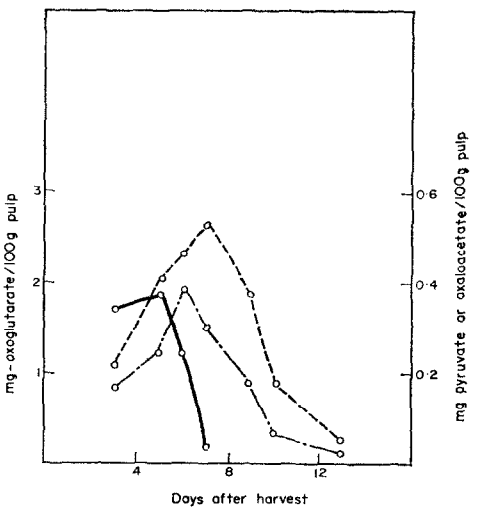


FIG. 2. CHANGES IN CONCENTRATION OF OXO ACIDS DURING RIPENING.
○——○ oxaloacetic acid; ○- - -○ α-oxoglutaric acid; ○-·-·-○ pyruvic acid.

acid respectively. Quantitative determination of the three oxo acids during the climacteric period gave the results shown in Fig. 2 which indicate that the concentration of these acids reach a maximum just before the respiration climacteric attains its peak value (Fig. 1). The maximum values are reached in a sequence in time: oxaloacetic acid (380 μg/100 g), pyruvic acid (390 μg/100 g), α-oxoglutaric acid (2800 μg/100 g).

By two dimensional paper chromatography 12 amino acids were shown to be present in extracts from mango pulp, 10 of which were identified by running authentic samples simultaneously. Changes in three of the acids, aspartic, glutamic and γ-aminobutyric were followed during the climacteric period and the results are shown in Fig. 3. From these results it would appear that the concentrations of all three amino acids had a minimum value as the respiration climacteric reached its peak, i.e. at about the same time as pyruvic and α-oxoglutaric acids were at maximum concentrations.

TABLE 1. R_f VALUES AND ABSORPTION MAXIMA OF 2:4 DINITROPHENYLHYDRAZONE DERIVATIVES OF α-OXO ACIDS EXTRACTED FROM MANGO PULP

	R_f values in solvent system* A		B		Absorption maximum (nm)	
	Observed	Reported	Observed	Reported	Observed	Reported
Pyruvate P1	0·41	0·46	0·54	0·44	450	445
P2	0·70	0·60	0·80	0·66		
Oxaloacetate	0·24	0·24	0·24	0·20	450	450
α-Oxoglutarate	0·14	0·18	0·35	0·30	380	432 (broad)

A = butanol–ethanol–0·5 N ammonia (7:1:2) and B = *t*-amyl alcohol–ethanol–water (5:1:4), both on buffered paper (phosphate, 0·2 M, pH 6·2).

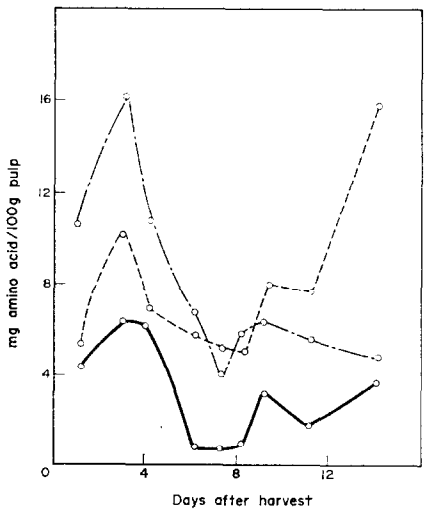


FIG. 3. CHANGES IN CONCENTRATION OF SOME FREE AMINO ACIDS DURING RIPENING.
○——○ aspartic acid; ○·····○ glutamic acid; ○- - -○ γ-aminobutyric acid.

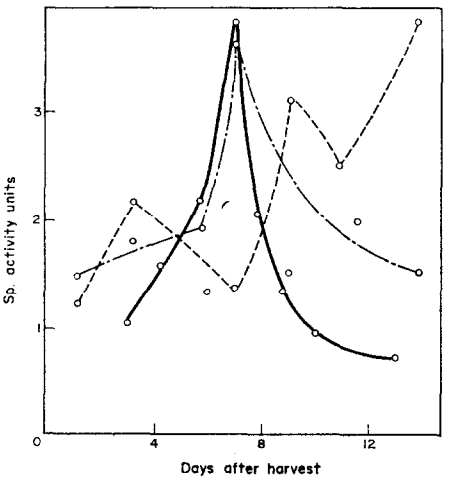
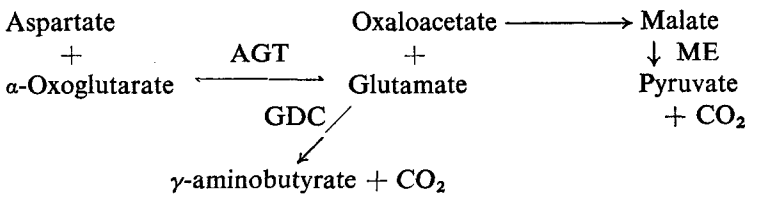


FIG. 4. CHANGES IN ENZYME ACTIVITY DURING RIPENING.
○——○ ME (decarboxylating) (sp. activity × 10) (specific activity units expressed as OD change at 340 nm/hr/mg protein); ○-·-·-○ AGT (specific activity units expressed as mg amino acid formed/30 min/mg protein); ○- - - -○ GDC (sp. activity units are divided by 10) (specific activity units expressed as μl CO_2/hr/mg protein).

The presence of ME, GDC, AGT, glutamic dehydrogenase and ABT was detected in the pulp but quantitative changes over the climacteric period were measured only for the first three of these enzymes. From the results shown in Fig. 4, it will be seen that the maximum activity of ME and AGT occurred just before the climacteric peak while the activity of GDC began to increase at this point and rose rapidly at the climacteric peak and beyond.

DISCUSSION

The possible transformations of oxo and amino acids through the action of some of the enzymes studied are shown in Scheme 1. According to this scheme, the action of AGT would result in a fall in aspartate and α-oxoglutarate with a corresponding rise in oxaloacetate and glutamate (or vice-versa). The rise in oxaloacetate could be offset by an immediate decarboxylation to pyruvate.



SCHEME 1. TRANSFORMATIONS OF OXO AND AMINO ACIDS IN MANGO.

During the rise in respiration to the climacteric peak there is, indeed, a fall in aspartate but at the same time α-oxoglutarate rises and glutamate is falling so that the increased activity of AGT during this phase (Fig. 4) cannot be related directly to transamination reactions between the oxo and amino acids. Again, during the phase following the climacteric peak (days 8 to 14) the changes in oxo acids, amino acids and AGT activity do not follow the course expected from Scheme 1. However, the rise in GDC activity (Fig. 4) accompanied by a fall in glutamic acid and a rapid rise in γ-aminobutyric acid (Fig. 3) form a pattern consistent with the scheme.

The rise in glutamic and aspartic acids following the climacteric peak, i.e. during the phase of rapid ripening, is similar to that found by Freeman and Woodbridge[7] and Davis[6] during the ripening of tomatoes. On the other hand, Mattoo and Modi[8] found a decrease in aspartic acid during the ripening of the mango.

The increased activity of the three enzymes during the climacteric rise agrees with the finding of Hulme *et al.*[2] for apples. Together with the fall in the concentrations of the amino acids during the early stages of the climacteric it is also consistent with the evidence for protein synthesis in apples[9] and pears[10] during this period, although such synthesis does not, apparently, occur in the avocado[11] or the banana.[12] The increased activity of ME and GDC could be contributing to the increased CO_2 production involved in the respiration climacteric.

EXPERIMENTAL

Mature fruits of the *Pairi* variety were collected from the local orchards. Fruits of uniform size and of the same physiological age were selected and stored in crates at room temperature (28°). Three or 4 fruits were analysed individually at regular intervals. Fruits respiring at the same rate as judged by CO_2 release were selected at each point for study. All the studies were carried out with the pulp of the fruit. The experiments were conducted during the seasons of 1964–68.

Respiration of the whole fruit. This was measured at 28° as described previously.[13]

Isolation, identification and estimation of oxo acids: extraction. 50·0 g of mango pulp was blended with 100 ml ice cold H_2O for 5 min., centrifuged and the supernatant collected. The residue was resuspended in H_2O and centrifuged. From this extract 2:4 dinitrophenyl hydrazones of oxo acids were prepared and estimated as described earlier.[14] All measurements were carried out at 420 nm in a spectrophotometer. The individual hydrazones were identified by (a) absorption spectrum, (b) reduction to corresponding amino acids using platinum catalyst and identification of the amino acids formed and (c) R_f values of the hydrazones as compared with the standards (Table 1).

Estimation of amino acids. 25·0 g mango pulp was blended with EtOH (80%). The clear extract obtained by centrifugation was concentrated to 5 ml *in vacuo* at 37°. The concentrate was passed through a column (1·5 × 6 cm) of Dowex 50 H^+, washed with H_2O and the amino acids eluted with 25 ml of 2N NH_4OH. The eluate was concentrated to 1 ml. For qualitative assay 2-D chromatogram with *n*-BuOH–HOAc–H_2O (10:3:7) and phenol–H_2O (4:1), respectively was developed in an ascending manner. The amino acids were identified by running authentic samples simultaneously. Qualitative determination was made by chromatography on Whatman paper No. 1 in phenol–H_2O (ascending). Glutamate, aspartate and γ-aminobutyrate were estimated as described[15] by comparison with standards.

Preparation of the fraction for enzyme assay: extraction. 80·0 g mango pulp was homogenized in a Waring Blendor for 5 min with 30 ml of 0·25 M sucrose containing 0·1% polyvinylpyrrolidone (PVP 40) maintaining pH at 7·0. The clear supernatant obtained from this was treated after centrifugation at 10,000 *g*

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for 20 min with Pectinol R-10 (Rohm & Hass) for 20 min at 2° to remove the gel forming substances during enzyme fractionation. The above operations were carried out at 0–4°.

Ammonium sulphate fractionation. 0–60% saturation ammonium sulphate fraction was prepared. After dialysis against 0·02 M tris HCl buffer, pH 7·4, for 2 hr, this fraction was used for the assay of the following enzymes—ME,[16] AGT,[17] ABT[18] and GDC[19] as described in methods in Enzymology. Protein was determined by the method of Lowry *et al.*[20]

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Abbreviations: Malic enzyme ME; L-aspartate-oxoglutarate aminotransferase AGT; γ-aminobutyric-oxoglutaric aminotransferase ABT; L-glutamate 1-carboxylase GDC.